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EFFECT OF THE DRYING MEDIA ON THE SURVIVAL RATE OF MICROBES IN THE DRY ANTIPLAGUE VACCINE

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pages 274-277.

For the preservation of the viability of the microbe cell during its transformation into the anabiotic state (the process of drying by the sublimation method), and in anabiosis (in the process of prolonged storage), the external medium factors are of extreme importance.

One of the most essential factors of this medium in the drying and storage of live antiplague vaccine (as well as of many other live vaccines) is the protective medium or the drying medium. During a relatively short period of time a great number of them were proposed; they produce different effects depending on the type of microbes.

For drying live antiplague vaccine M. M. Faybich and R. V. Korneyev's saccharose-gelatin medium was adopted, which possesses a number of positive properties. The disadvantage of this medium, and of many other drying media, is the appreciable loss of microbe cells in the process of sublimation and the subsequent storage of vaccines in it.

In connection with the publication by B. I. Blankov and D. L. Klebanov (1961) of the monograph Primeneniye liofilizatsii v mikrobiologii (The Use of Lyophilization in Microbiology), in which all the requirements to be met by the drying media are stated in full, and many various prescriptions for them with the corresponding characteristics thereof are adduced, we performed a comparative study of the effect of some of them on the survival rate of microbe cells in the live antiplague vaccine of the Ye V strain (NIIEG;Nauchno Issledovatel'skiy Institut Eksperimental'noy Gigiyeny; Scientific Research Institute of Experimental Hygiene).

For the above purpose we used the following protective drying media: 1. M. M. Faybich and R. V. Korneyev -- saccharose 10% + gelatin 1.5%; 2. B. I. Blankov and Yu. L. Subbotina (1960) -- gelatose 5% + saccharose 7.5%; 3. B. I. Blankov GMS -- saccharose 7.5% in the hydrolyzate of natural fat-free milk; 4. Hornybrook (1955) -- lactose-salt: potassium citrate 1.35 + sodium citrate 2.45 grams + potassium phosphate 0.61 gram + calcium chloride 1.33 grams + magnesium chloride 0.6 g. + potassium carbonate 1 gram + lactose 57.5 grams + water 1 liter (pH of the medium is 7.0). The medium is characterized by good solubility and absence of antigenic properties. 5. Fray -- glucose 7.5% + nutrient broth 25% + dextrin 5%; 6. Magleton (1960), recommended by him for drying BCG: saccharose 7.5% + dextrin 8% + sodium glutomate 2%; 7. Grieves (1960) -- sodium glutomate 5% + dextrin 5%.

The Ye V vaccine strain was used to inoculate Huttinger's agar (pH -- 7.2) with amine nitrogen content of 120-140 mg%.

The culture for the experiments was washed from two plates with one of the above media. The microbe suspension was kept for three days at 2°C (control for sterility), then the standard and the percentage of live microbes in it were determined. The drying was performed in the IIL-3 chamber apparatus according to the adopted drying procedure. The experiment with media 1, 2, and 4 was performed 6 times, with the others, because of the poor survival rate of the microbes in the vaccine, three times (Table 1).

Table 1

Effect of Various Drying Media on the Survival Rate of Microbes in Live Antiplague Vaccine in the Process of Lyophilization

(1) Среда высушивания	(2) Число опытов	(3) Содерж. микробных тел в вакцине			
		(4) жидкой		(5) сухой	
		стандарт млрд. м.т. (6)	% живых м. т. (7)	стандарт млрд. м.т. (8)	% живых м. т. (9)
8 Файбича	6	81	50	52	31,8
9 Бланкова (желатозная)	6	116	44	83	27,2
10 Бланкова (ГМС)	3	100	34,4	60	13
11 Хорнибрука	6	107	40,5	78	20,7
12 Фрая	3	90	21,5	57	3,3
13 Маглетона	3	98	23	65	0,3
14 Гривса	3	93	54	73	1,3

LEGEND: 1) Drying medium; 2) number of experiments; 3) microbe body content in the vaccine; 4) liquid; 5) dry; 6) standard billion m. b.; 7) % live m. b.; 8) Faybich; 9) Blankov (gelatose); 10) Blankov (GMS); 11) Hornybrook; 12) Fray; 13) Magleton; 14) Grieves.

As we can see from Table 1, the decrease of the survival rate of the microbes in the vaccine in the process of lyophilization proceeds along two lines: a) decrease of the vaccine's standard, which we tend to attribute mainly to the destruction of the live microbe cells during a specific phase of growth with a high osmotic pressure within the cell; and b) death of the microbe cells without breakdown thereof, occurring both in the liquid vaccine during the control period and in the process of drying.

Here the microbe survival rate is affected appreciably by the drying medium, beginning with the moment they are washed down. Thus, if the number of microbes suspended in the Faybich medium, after three days' refrigerated storage at 2°C is to be taken as 100%, then in the other media it will be the following:(Table 2).

Table 2

Effect of Various Drying Media on the Survival Rate of Microbes in the Culture Suspension

Среда (1)	Файбича (2)	Бланкова (желатозная) (3)	ГМС (4)	Хорнибрука (5)	Фрая (6)	Маглетона (7)	Гривса (8)
(9) % живых микробов	100	88	68,8	81	43	46	102

Legend: 1) Medium; 2) Faybich; 3) Blankov (gelatose); 4) GMS; 5) Hornybrook; 6) Fray; 7) Magleton; 8) Grieves; 9) % of live microbes.

From the above we can see that Grieves' medium is the most favorable for the survival of the Ya V microbes in the suspension. The other media decrease the number of live microbes in comparison with the Faybich medium, even before drying: gelatose -- by 12, Hornybrook -- by 19, GMS -- by 21, Magleton -- by 54, and Fray medium -- by 57%. The microbes die at an especially high rate in Magleton's and Fray's media.

However, although in Grieves' medium the standard and viability of the microbes is well preserved in the vaccine before drying, upon lyophilization, the highest percentage (77.4%) of the death of microbes is observed in it (Table 3).

From Table 3 it follows that the percentage of the total yield of live microbes in the dry vaccine, calculated with respect to the total amount of microbes suspended in the medium, varies greatly for various media. The optimum media are those of Faybich (20.3%) and Blankov (gelatose -- 19.5%). Hornybrook's medium occupies the third place. The remaining media do not protect the live vaccine from death during lyophilization.

Table 3

Effect of the Drying Medium on the Yield of Live Microbes in the Live Antiplague Vaccine

	(1) Среда высушивания	Выход живых микробов в сухой вакцине (в % от числа их во взвеси) (2)	(3) Отход их при лиофилизации, %		
			(4) за счет снижения стандарта	(5) за счет гибели микробов	(6) суммарный отход, %
7	Файбича	20,3	35,8	43,9	79,7
8	Бланкова (с желатозой)	19,5	28,2	52,3	80,5
9	ГМС	7,8	40,0	52,2	92,2
10	Хорнибрука	15,1	27,0	57,9	84,9
11	Фрая	2,1	36,6	61,3	87,9
12	Маглетона	0,2	33,7	66,1	99,8
13	Гривса	1,1	21,5	77,4	98,9

LEGEND: 1) Drying medium; 2) yield of live microbes in the dry vaccine (in % of their number in the suspension); 3) loss thereof upon lyophilization; 4) through lowering of the standard; 5) through death of the microbes; 6) total loss, %; 7) Faybich; 8) Blankov (with gelatose); 9) GMS; 10) Hornybrook; 11) Fray; 12) Magleton; 13) Grieves.

The change in the number of live microbes in the vaccine in the process of storage is given in Table 4.

The best results with respect to the survival rate of the microbes after 4 month of storage of live antiplague vaccine are obtained with Grieves' medium (no decrease), however, as we have mentioned, the initial percentage of live microbes after lyophilization in it was extremely low, which fact diminishes its merits. Taking this into account we conclude that the most advantageous medium (the survival rate during the same time decreases only 9.4%) is Faybich's protective medium, with which, according to A. N. Kraynova et al. (1960) and N. F. Bystryy et al. (1961), the period of usability of the vaccine can be prolonged to 2-3 years, which fact is accepted by the new instruction. With the other protective media studied, we note a sharp (from 31 to 100%) decrease of survival of microbes in the vaccine after a storage period of 4 months only.

The analysis of the data obtained by us gave us an idea on the rationality of studying the effectiveness of combination in the drying medium of individual ingredients of the Grieves and Faybich media. For this we performed experiments with 3 new series of the vaccine. The following results were obtained (Table 5).

From this Table we see that the addition to the Faybich and Korneyev medium of 5% of sodium glutomate increases the yield of live microbes in the dry vaccine, albeit to a small degree (3.6%), whereas the addition of 7.5% sodium glutomate

Table 4

Survival Rate of Microbes in the Vaccine in the Process of Storage

(1) Среда высушивания	(2) Процент выживаемости исходной сухой вакцины	(3) Снижение его при хранении в теч. (4) месяца	(5) 4 месяцев	(6) % относит снижения к концу 4 мес.
7 Файбича . . .	31,6	30,3	28,8	9,4
8 Бланкова (желатозная)	27,2	18,5	15,9	42
9 ГМС . . .	13	10	8,5	35
10 Хорнибрука . . .	20,7	20	14,3	31
11 Фрая	3,3	1,5	0,5	85
12 Маглетона	0,3	0,05	0	100
13 Гривса	1,3	1,25	1,6	0

LEGEND: 1) Drying medium; 2) percentage of survival of the initial dry vaccine; 3) its decrease upon storage for; 4) one month; 5) four months; 6) % of relative decrease by the end of four months; 7) Faybich; 8) Blankov (gelatose); 9) GMS; 10) Hornybrook; 11) Fray; 12) Magleton; 13) Grieves.

or 5% dextrin result in the decrease of these indexes.

Thus, the study of the effect of various drying media and several combinations thereof, showed that the best medium for drying live antiplague vaccine is the Faybich and Korneyev medium; upon addition to it of 5% of sodium glutomate it would possibly yield still more stable results. The second place is occupied by the protective gelatose Blankov medium

Table 5

Effectiveness of the Combination of the Faybich Drying Medium With the Ingredients of the Grieves Medium

(1) Варианты сочетаний сред высушивания	(2) Среднее число микробов (в млрд.) в 1 мл среды до высушивания	(3) Сухая вакцина: (4) Число живых микробов в 1 мл, в млрд.	(5) Процент выхода живых микробов
6 Среда Файбича	90	29	32,2
7 Среда Файбича + глутомат натрия 5%	85	30,4	35,8
8 Среда Файбича + глутомат натрия 7,5%	88	26	29,5
9 Среда Файбича + декстрин 5%	87	22,6	26

LEGEND: 1) Variations of drying media combinations; 2) mean number of microbes (in billions) per 1 ml of medium before drying; 3) dry vaccine; 4) number of live microbes in 1 ml, in billions; 5) percentage of yield of live microbes; 6) Faybich medium; 7) Faybich medium + sodium glutomate 5%;

8) Faybich medium + sodium glutomate 7.5%; 9) Faybich medium + dextrin 5%.

and the lactose-salt Hornybrook medium. The rest of the adduced media are unsuitable for drying the antiplague vaccine.

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